

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Peled et al.	CONF No.:	9770
SERIAL NUMBER:	10/774,843	EXAMINER :	Maria Gomez Leavitt
FILING DATE:	February 9, 2004	ART UNIT :	1633
FOR:	EXPANSION OF RENEWABLE STEM CELL POPULATIONS		

Via EFS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. TONY PELED UNDER 37 C.F.R. §1.132

I, Tony Peled, declare and state that:

1. I received a Ph.D. degree from the Hebrew University - Hadassah Medical School in Jerusalem, Israel. I am the Chief Scientist, Vice President and co-founder of Gamida Cell Ltd. of Jerusalem, Israel (the Assignee of the above-referenced application). A principal aspect of my research is the study of stem cell culture and the therapeutic application of stem cell technology. I am the author of numerous peer-reviewed publications and posters, most of which are directed to cell expansion and cell differentiation, with specific focus on hematopoietic stem cells.
2. I have reviewed the Final Office Action dated April 30, 2010. I understand that claims 401, 411, 414, 416, 419, 422-424, 464, 465, 469-471 and 478-480 are rejected under 35 U.S.C. § 103(a) as being unpatentable over US Patent Publication No. 2002/0159984 to Brown ("Brown") over U.S. Patent No. 6,413,772 to Block ("Block").
3. I have reviewed the accompanying amendment and the above-referenced application in conjunction with the cited references.
4. I assert that CD34+ hematopoietic stem cells of the instant invention are cultured *ex-vivo* under conditions allowing for cell proliferation. These conditions require providing

nutrients, serum, and a combination of cytokines including each of stem cell factor, thrombopoietin, FLT3 ligand and IL-6. The CD34⁺ hematopoietic stem cells of the present invention can also be cultured under these conditions and further include IL-3.

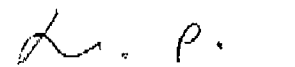
5. The data accompanying this declaration shows the effect of nicotinamide (NAM) in CD34⁺ cells derived from umbilical cord blood during 3 weeks in cultures supplemented with cytokines (each of FLT3, IL-6, TPO, and SCF). Analysis included the number of total nucleated cells (TNC), colony forming unit cells (CFUc) and phenotypic characterization of hematopoietic progenitors, CD34⁺ and CD34⁺CD38⁻ cells.
6. Figure 1 accompanying this declaration shows cord blood-derived purified CD34⁺ cells cultured for 3 weeks with cytokines (each of FLT3, IL-6, TPO, and SCF) or with cytokines (each of FLT3, IL-6, TPO, and SCF) and nicotinamide ("NAM") at 2.5 and 5 mM. Each bar represents the average \pm SE of 4 independent experiments.
7. Panel A-E shows the analysis of cultured cells one week post seeding.
8. Panel A shows the number of total nuclear cells (TNC) (*P<0.01 vs. NAM, 2.5 and 5 mM).
9. Panel B shows the number of CD34⁺ cells (*P<0.008 vs. NAM).
10. Panel C shows representative FACS analysis dot plots of cells double stained with CD34 PE and CD38 FITC.
11. Panel D shows percentages of CD34⁺CD38⁻ cells (*P<0.01 vs. NAM non-treated cultures).
12. Panel E shows the numbers of CD34⁺CD38⁻ cells (*P<0.03 vs. NAM non-treated cultures).
13. To track cell division history, freshly purified CD34⁺ cells were labeled with PKH2, cultured and analyzed 7 day post seeding. Histograms of PKH fluorescence intensity of CD34⁺ (Panel F) and CD34⁺CD38⁻ (Panel G) cells are shown. The histograms present the same number of cells for both control and NAM-treated cells in a representative experiment out of three experiments performed.

14. Panel H shows the median fluorescence intensities of NAM expanded cells on day 7 cultures of three separate experiments as percentages of control cultures treated with cytokines alone.
15. Panel I shows the percentages of $CD34^+CD38^-$ cells, one, two and three weeks post seeding ($*P \leq 0.01$ vs. NAM non-treated cultures). Panels J-L show the fold expansion (FE) of TNC ($*P < 0.01$ vs. NAM, $**P < 0.03$ vs. NAM 5mM) (J), CFUc ($*P < 0.03$ vs. NAM, $**P < 0.02$ (K) and $CD34^+$ cells ($*P \leq 0.01$ vs. NAM) (L), one, two and three weeks post seeding.
16. Figure 2 accompanying this declaration shows cord blood derived purified $CD34^+$ cells cultured with cytokines, with and without NAM (2.5-5mM) and FACS analyzed after 3 weeks as following: cells were count and thereafter stained with FITC-conjugated antibodies against differentiation antigens (CD38, CD33, CD14, CD15, CD3, CD61, CD19) and with PE-conjugated antibodies against CD34. Positive PE and negative FITC cells were considered as $CD34^+Lin^-$ cells. The results show the actual number in culture of $CD34^+CD38^- Lin^-$ cells calculated from the total number of cells. Each experiment was repeated with cells derived from 6 different cord blood units.
17. The data accompanying this declaration shows, as early as one week post seeding, that the number of total nuclear cells (TNC) (Fig. 1A) and $CD34^+$ cells (Fig. 1B) were substantially lower, while percentages (Fig. 1C-D) and absolute number (Fig. 1E) of $CD34^+CD38^-$ cells were significantly higher in cultures treated with 2.5 and 5 mM NAM as compared with control cultures treated with cytokines only. The divisional history of seeded $CD34^+$ cells stained with PKH indicated that during the first week in culture, the vast majority of $CD34^+$ cells underwent several cycles under both culture conditions (Fig. 1F-G), with consistent lesser divisions (higher fluorescence intensity) of cells cultured with NAM (Fig. 1H). Slower cycling was particularly prominent in the $CD34^+CD38^-$ subset (Fig. 1G) which, nevertheless, increased within the expanded cell population from week one through week-3 (Fig. 1I). After three weeks in culture, fold expansion of TNC (Fig. 1J), CFUc (Fig. 1K) and $CD34^+$ cells (Fig. 1L) in NAM-treated reached the values in NAM non-treated cultures. Phenotype characterization of lineage differentiated cells in three week cultures revealed lessening of differentiation in cultures treated with NAM (2.5 and 5 mM) than in cultures treated with

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cytokines alone, as demonstrated by significant lower percentages of CD14, CD11b, CD11c and CD15⁺ cells (Fig. 2).

18. Thus, it is shown that CD34⁺ cells cultured with NAM displayed an initial slower proliferation rate than their counterparts cultured without NAM. This was most pronounced in the putative HPC compartment defined as CD34⁺CD38⁻ cells, along with an increase in their numbers in the presence of NAM. Our results demonstrate that culturing with each of FLT3, IL-6, TPO, and SCF in the presence of NAM produced an expanded CD34⁺ hematopoietic stem cell population with an increased proportion of CD34⁺/Lin⁻ and CD34⁺/CD38⁻ cells in the expanded culture as compared to CD34⁺ cells cultured in the presence of those cytokines and nutrients without exogenously added nicotinamide.
19. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.


Lony Peled, Ph.D.

Signed this 30 day of July, 2010

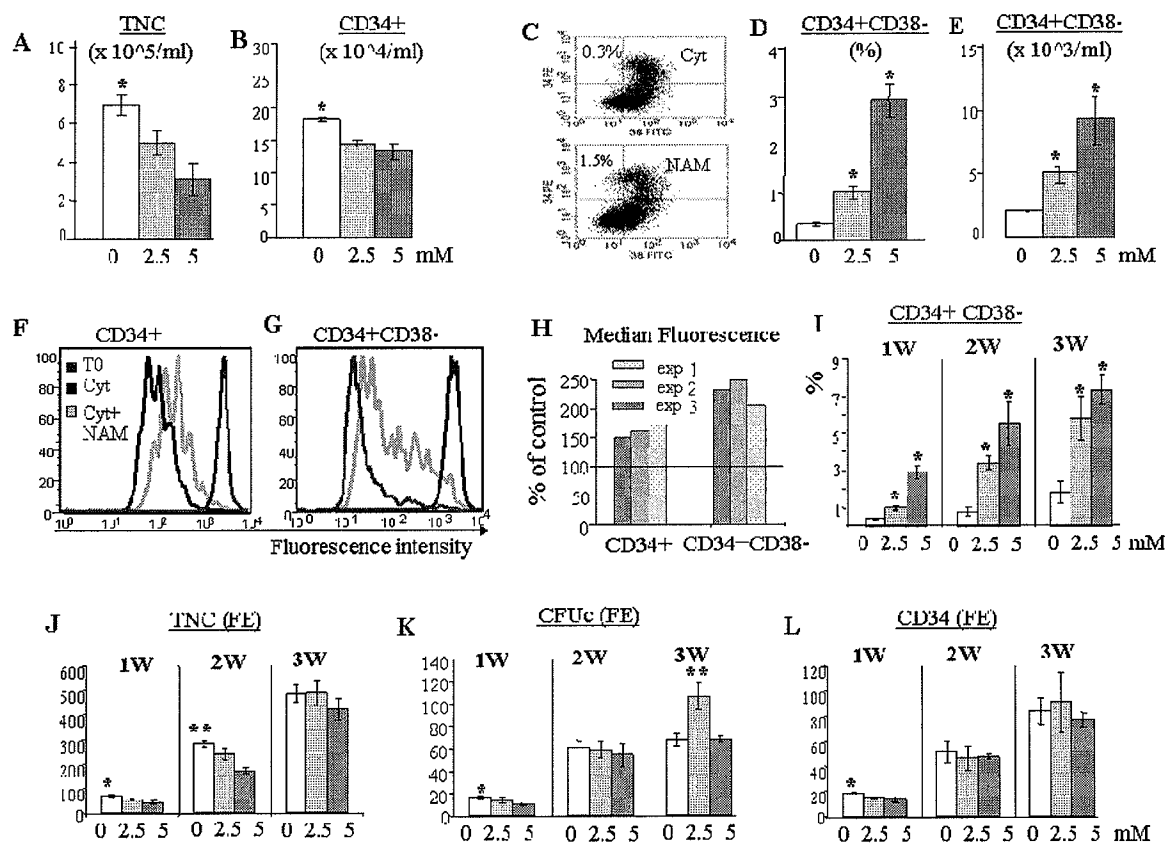


Fig.1

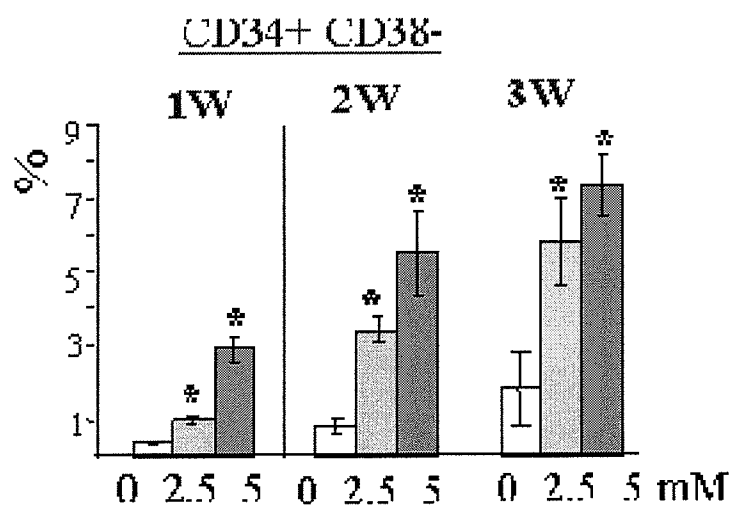


Figure 1

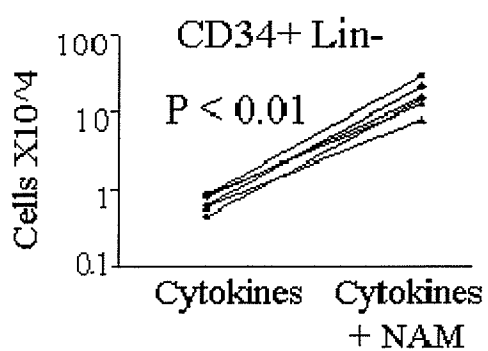


Figure 2

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